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Fully-automated synthesis of 16β-^{18}F-fluoro-5α-dihydrotestosterone (FDHT) on the ELIXYS radiosynthesizer

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HIGHLIGHTS

- Manual radiosynthesis of ^{18}F-FDHT was adapted for full automation on the ELIXYS radiosynthesizer.
- Reduction with $LiAlH_4$ was performed at room temperature to avoid cryogenic conditions.
- Formulated product passed all clinical QC tests and is suitable for clinical production.
- Decay-corrected radiochemical yield was $29 \pm 5\%$ ($n=7$) with a synthesis time of 90 min.
- Specific activity was 4.6 Ci/μmol (170 GBq/μmol) at the end of formulation.

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ABSTRACT

Noninvasive *in vivo* imaging of androgen receptor (AR) levels with positron emission tomography (PET) is becoming the primary tool in prostate cancer detection and staging. Of the potential ^{18}F-labeled PET tracers, ^{18}F-FDHT has clinically shown to be of highest diagnostic value. We demonstrate the first automated synthesis of ^{18}F-FDHT by adapting the conventional manual synthesis onto the fully-automated ELIXYS radiosynthesizer. Clinically-relevant amounts of ^{18}F-FDHT were synthesized on ELIXYS in 90 min with decay-corrected radiochemical yield of $29 \pm 5\%$ ($n=7$). The specific activity was 4.6 Ci/μmol (170 GBq/μmol) at end of formulation with a starting activity of 1.0 Ci (37 GBq). The formulated ^{18}F-FDHT yielded sufficient activity for multiple patient doses and passed all quality control tests required for routine clinical use.

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1. Background

Prostate cancer has become and remains the second leading cause of cancer-related death in American men (CDC, 2014). Effective management of prostate cancer requires early detection and the availability of accurate diagnostic modalities for predicting and monitoring the disease (Liu et al., 1992a). Increased androgen receptor (AR) expression in primary tumors of prostate cancer are strong indicators of the disease; however, due to heterogeneity of the tumors, biopsy samples alone may not be sufficient for disease detection (Beattie et al., 2010; Liu et al., 1992b). Molecular imaging agents that can noninvasively provide prognostic information for distinguishing AR-positive tumors are critically important for the treatment of prostate cancer (Brandes and Katzenellenbogen, 1987). Over the years, a number of fluorinated androgen derivatives have been synthesized and evaluated for AR-binding and tissue distribution *in vivo* using positron emission tomography (PET) (Choe et al., 1995; Liu et al., 1992a). Several promising candidates have been successfully labeled with the positron emitting radionuclide fluorine-18 in efforts to develop PET tracers for tumor localization in patients with metastatic prostate cancer (Liu et al., 1992b). 16β-^{18}F-fluoro-5α-dihydrotestosterone (^{18}F-FDHT), a fluorinated analog of the native AR-binding ligand dihydrotestosterone, has been studied in both rat (Liu et al., 1992b) and

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primate models (Bonasera et al., 1996) and has proven to be one of the most effective *in vivo* AR-binding radiotracers studied to date.

Significant interest in evaluating and monitoring AR expression in prostate cancer patients has led to multiple clinical studies using ^{18}F-FDHT. The overall goal of these studies was to assess the potential of ^{18}F-FDHT as a diagnostic tool for imaging AR expression in prostate cancer patients. In these studies, ^{18}F-FDHT was found to selectively bind AR in both primary tumor and metastatic sites suggesting its crucial role in prostate cancer imaging (Beattie et al., 2010; Bonasera et al., 1996; Dehdashti et al., 2005). An extensive clinical study conducted by Beattie et al. (2010) evaluated the pharmacokinetic properties of ^{18}F-FDHT and found that, indeed, uptake of the tracer in prostate tumors correlated reasonably well with AR expression in metastatic prostate cancer. Clinical efficacy proved promising, even when compared to ^{18}F-FDG in patients with castration-resistant prostate cancer (CRPC) (Larson et al., 2004). A more recent study compared the features of bone metastases in 38 CRPC patients to ^{18}F-FDG and ^{18}F-FDHT uptake as well as overall survival. This study concluded that the degree of androgen receptor expression, measured by the intensity of ^{18}F-FDHT uptake, and the number of bone lesions, were significantly associated with patient survival (Vargas et al., 2014). These studies and others have established ^{18}F-FDHT as an important tracer in the management of advanced prostate cancer; ^{18}F-FDHT is now being applied in breast cancer (NCT01988324), primary prostate cancer (NCT02297386) and is the focus of an international multi-center clinical trial in the USA, Europe and Australia (NCT00588185).

As more clinical studies are conducted, the ability of ^{18}F-FDHT to elucidate the role of AR expression in metastatic prostate cancer is becoming clearer. In addition to providing prognostic information, ^{18}F-FDHT is a critically important biomarker used to accelerate research towards the treatment of CRPC and, ultimately, for the clinical management of the disease. Although not all patients respond, drugs targeting AR signaling represent tremendous advances in the treatment of the lethal phase of CRPC (de Bono et al., 2011; Scher et al., 2012). ^{18}F-FDHT PET can act as a noninvasive tool to measure the pharmacodynamic response of an AR-binding substrate; such is the case with ARN-509 (Rathkopf et al., 2013). As a second generation antiandrogen, ARN-509 recently demonstrated favorable safety, tolerability, pharmacokinetics, pharmacodynamics and antitumor activity in men with CRPC in phase I clinical trials. Measurements based on ^{18}F-FDHT PET confirmed on-target effect and maximal AR inhibition, ultimately guiding the patient dose chosen for Phase II clinical trials.

The synthesis of ^{18}F-FDHT was reported by Liu et al. (1992b), via the precursor 16α-[[(trifluoromethyl)sulfonyl]oxy]-3,3-(ethylenedioxy)androstan-17-one **1** (Scheme 1). Nucleophilic displacement of the triflate of precursor **1** with nBu$_4$^{18}F gave the fluorinated intermediate **2**, which underwent subsequent reduction via lithium aluminum hydride ($LiAlH_4$) to afford the α-hydroxyl intermediate **3**. Acid-catalyzed deprotection of the ketal **3** yielded the desired compound, ^{18}F-FDHT, in three total steps. The total synthesis time for the manual process, including high-performance liquid chromatography (HPLC) purification, but not including reformulation, was 90 min. The reported decay-corrected radiochemical yield (RCY) was 31–48%, and the specific activity was 1.2 Ci/μmol (43 GBq/μmol). The manual production of ^{18}F-FDHT currently employed in Memorial Sloan Kettering Cancer Center (MSKCC) for clinical trials is based on this synthetic protocol. The decay-corrected RCY obtained currently at MSKCC is 44 ± 5% (n=4) with a synthesis time (that includes reformulation) of about 110 min.

The demand for ^{18}F-FDHT is expected to increase as the clinical potential of this PET tracer is being recognized (Rice et al., 2011), thus an automated synthesis of this tracer will be essential for reliable, routine production. Currently, the clinical production of ^{18}F-FDHT is performed manually by trained radiochemists; as such, its use is limited to a few sites. Automation of this synthesis would enable many more facilities currently equipped for PET synthesis to routinely obtain ^{18}F-FDHT without the need for specially-trained personnel. We have automated the synthesis of ^{18}F-FDHT on the ELIXYS radiosynthesizer without the need for substantial modification of the synthesis approach. Our primary goal was to quickly translate the accepted manual synthesis currently in place at MSKCC onto an automated platform for immediate use in clinical trials. Herein, we describe the first demonstration of an automated synthesis of ^{18}F-FDHT.

2. Methods

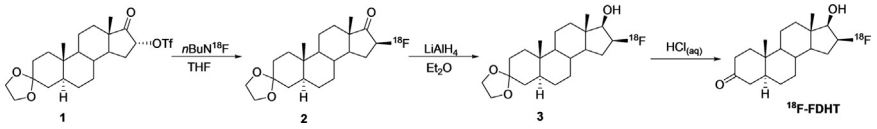
2.1. Materials

2.1.1. Reagents

No-carrier-added ^{18}F-fluoride was produced by the (p,n) reaction of ^{18}O–H_2O (84% isotopic purity, Medical Isotopes; Pelham, NH, USA) in a RDS-112 cyclotron (Siemens; Knoxville, TN, USA) at 11 MeV using a 1 mL tantalum target with havar foil. All commercially available reagents were used as received unless otherwise specified. Anhydrous solvents were obtained by filtration through activated alumina columns. Sodium sulfate anhydrous (Na_2SO_4; granular, EMD Chemicals), magnesium sulfate anhydrous crystalline ($MgSO_4$; MP Biomedicals) and HPLC grade dichloromethane (DCM) were obtained from Fisher Scientific (Pittsburg, PA, USA). HPLC grade ethyl acetate (EtOAc), HPLC grade acetonitrile (MeCN), anhydrous hexane, 1.0 M $LiAlH_4$ in diethyl ether, and tetrabutylammonium hydroxide (~40% in water) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). USP grade sterile saline was obtained from Hospira, Inc. (Lake Forest, IL, USA). 200-proof ethanol (EtOH) was purchased from the UCLA Chemistry Department (Los Angeles, CA, USA). FDHT precursor and cold standard were prepared as previously described (Liu et al., 1992b) at the MSKCC Organic Synthesis Core. The precursor solution used in each synthesis contained 8 mg of the precursor dissolved in 0.5 mL of anhydrous THF. $LiAlH_4$ solution was loaded into a reagent vial under an inert atmosphere of nitrogen in an mBraun UNILAB glovebox (Stratham, NH, USA). All water used was purified to 18 MΩ and passed through a 0.1 μm filter.

2.1.2. Cartridges

HLB cartridges were purchased from Waters (Milford, MA, USA) and preconditioned with 5.0 mL of EtOH, 10.0 mL of water, and dried with nitrogen. Preconditioned quaternary methylammonium (QMA) cartridges were purchased from ABX (K-920, Advanced Biochemical Compounds; Radeberg, Germany) and used as received. Drying cartridges were made in-house by filling empty polypropylene solid-phase extraction tubes, fitted with



Scheme 1. Three-step synthesis of ^{18}F-FDHT.

polypropylene frits (57024, Sigma-Adrich; Milwaukee, WI, USA; 20 μm pore size), with either 2.0 g of Na_2SO_4 or 2.0 g of $MgSO_4$ and capped with syringe adapters (210705, Grace; Columbia, MD, USA). For each synthesis, one of each cartridge was used. Sterile syringe filters (PVDF membrane) were purchased from Fisher Scientific (SLGVM33RS, 0.22 μm pore size, 33 mm diameter) and used for the final reformulation.

2.2. Chromatography

Semi-preparative HPLC (10 mL/min flow rate of 7% EtOAc in DCM) was performed with a WellChrom K-501 HPLC pump (Knauer; Berlin, Germany), normal-phase Luna column (5 μm, 21.2 × 100 mm^2, Phenomenex), ultraviolet (UV) detector (254 nm, WellChrom Spectro-Photometer K-2501, Knauer), and gamma-radiation detector and counter (B-FC-3300 and B-FC-1000; Bioscan Inc.; Washington, DC, USA). Analytical HPLC (1 mL/min flow rate of 55% MeCN in water) was performed on a Knauer Smartline HPLC system with a Phenomenex reverse-phase Gemini column (5 μm, 4.6 × 250 mm^2) with inline Knauer UV (190 nm) and gamma-radiation coincidence detector and counter (B-FC-4100 and B-FC-1000). HPLC chromatograms were collected by a GinaStar (raytest USA, Inc.; Wilmington, NC, USA) analog to digital converter and GinaStar software (raytest USA, Inc.) running on a PC. Specific activity values were determined by dividing the activity amount injected into analytical HPLC by the injected mass as calculated from a standard curve using ^{19}F-FDHT. Radio-thin-layer chromatography (radio-TLC) was performed on a miniGita Star (Raytest USA, Inc.) using precut silica plates (Baker-flex; J.T. Baker) developed in 10% EtOAc in hexane (v/v). Samples taken for radio-TLC after $LiAlH_4$ reduction were immediately quenched in acetone prior to development and analysis.

2.3. Radiosynthesizer

Synthesis was performed using an ELIXYS automated radiosynthesizer (Lazari et al., 2013) (Sofie Biosciences, Inc.; Culver City, CA, USA) at the Crump Cyclotron and Radiochemistry Technology Center in the Crump Institute for Molecular Imaging at UCLA.

2.4. Cassette setup

The synthesis was performed using two reactors and cassettes. Reactor 1 was used for all reaction and evaporation steps, and all reagents were loaded into Cassette 1 (Table 1). The ^{18}F-fluoride source vial was connected to the radioisotope input of Cassette 1, and a QMA cartridge was installed in Cartridge Position #1. An HLB cartridge was installed in Cartridge Position #2 for solid-phase extraction and solvent exchange (i.e., from aqueous to organic phase) prior to HPLC purification. The output of this pathway was connected to an internal three-way valve to transfer fluid to either a waste container or a Na_2SO_4 cartridge, followed by a $MgSO_4$ cartridge, and finally to the direct-input of Cassette 2. Cassette 2 was used simply to load the crude product into the HPLC loop for purification. An illustration of the fluid diagram is shown in Fig. 1.

Table 1
List of reagents as organized on Cassette 1.

Position	Reagent
1	6.5 μL of ~40% TBAH with 0.5 mL water and 0.5 mL MeCN
2	1.0 mL MeCN
3	1.0 mL MeCN
4	8.0 mg precursor in 0.5 mL THF
5	0.35 mL of 1.0 M $LiAlH_4$ in diethyl ether
6	0.10 mL acetone in 0.25 mL THF
7	1.0 mL of 3.0 N HCl
8	3.0 mL of water
9	3.0 mL of water
10	3.0 mL of water
11	2.5 mL DCM
12	2.5 mL DCM

2.5. Synthesis protocol

^{18}F-fluoride handling was performed as previously reported (Lazari et al., 2014). Briefly, ^{18}F-fluoride in water was delivered into Cassette 1 using positive pressure (6 psig) through the QMA cartridge. Trapped ^{18}F-fluoride was subsequently eluted with TBAH eluent solution (Reagent Position 1, Table 1) into Reactor 1. Contents were partially evaporated while applying both vacuum and a stream of nitrogen (10psig) at 110 °C for 4 min without stirring. Acetonitrile (Reagent Position 2) was added through the QMA cartridge (3 psig driving pressure) to wash any remaining activity into Reactor 1, and the combined contents of the reactor were similarly evaporated for 2 min. Acetonitrile (Reagent Position 3) was then directly added to Reactor 1, and the contents were fully evaporated using previous conditions for 2 min. Reactor 1 was cooled to 30 °C, and the precursor solution (Reagent Position 4) was then added. Contents were reacted at 75 °C for 5 min with stirring. Once the reaction was complete, the solution was cooled to room temperature, and the $LiAlH_4$ solution (Reagent Position 5) was slowly added (1 psig driving pressure), followed by 20 s of stirring. The reduction reaction was quenched via the slow addition (1 psig driving pressure) of an acetone–THF solution (Reagent Position 6), and the solution was stirred for another 20 s. HCl (Reagent Position 7) was added, and the solution was reacted at 75 °C for 10 min with stirring. The reactor was cooled, and the contents were diluted with water (Reagent Position 8). The contents were transferred through the HLB cartridge (5psig driving pressure), where the product was trapped and highly polar components such as ^{18}F-fluoride passed through to a waste vial. Reactor 1 and the HLB cartridge were rinsed with two more volumes of water (Reagent Positions 9–10; 5 psig driving pressure). The HLB cartridge was dried with nitrogen at 15 psig for 1.5 min. Reactor 1 was filled with DCM (Reagent Position 11), which was transferred through the HLB cartridge (10 psig driving pressure) and subsequently through the Na_2SO_4 and $MgSO_4$ cartridges; this process elutes the ^{18}F-FDHT from the HLB cartridge and removes residual water before delivering the dry contents into a vial in Reactor 2. This process was repeated once more with DCM (Reagent Position 12), followed by the application of 15psig of nitrogen gas for 45 s to ensure all liquid was transferred to Reactor 2. The ^{18}F-FDHT product was purified by HPLC by remotely loading the crude solution into the HPLC loop provided on the ELIXYS and injecting the contents into the HPLC.

2.6. Reformulation

The final product was formulated in a ~8% ethanol in sterile saline solution. A remote-controlled fraction collector delivered the purified fraction of ^{18}F-FDHT into our custom rotary evaporator fitted with a 100 mL glass pear flask. Contents were fully evaporated, dissolved with 0.5 mL of EtOH, and diluted with 6.0 mL of sterile saline. The final formulation was remotely transferred into a sterile vial through a 0.22 μm sterile filter.

2.7. Quality control (QC)

The radiochemical purity and identity of the obtained product was confirmed by performing reverse-phase HPLC. The retention time for the radioactive peak produced by the product was compared to the FDHT reference standard peak retention time to

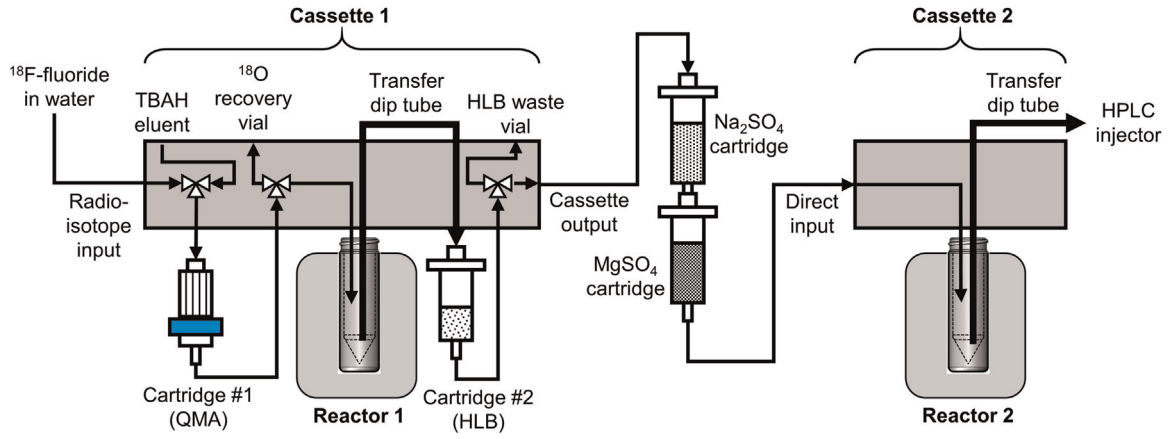


Fig. 1. Fluid diagram and cassette setup for automated ^{18}F-FDHT synthesis. All components within the grey rectangles (i.e., three-way stopcock valves, transfer dip tubes, and the necessary fluid connection ports) are pre-installed in the disposable cassettes. Both cassettes are identical in construction and internal components, but unused components are omitted from Cassette 2 for clarity.

confirm the radiochemical identity. Additional testing included visual inspection, pH, radionuclidic identity via half-life determination, filter integrity testing, determination of residual solvents by gas chromatography, endotoxin level determination and sterility. The obtained results conform to the FDHT acceptance specifications established at MSKCC Radiochemistry and Molecular Imaging Probes Core for routine manual production of ^{18}F-FDHT.

3. Results

Decay-corrected RCY was $29 \pm 5\%$ ($n=7$) and was determined by dividing the decay-corrected reformulated activity by the initial starting activity. After automation of the synthesis, validation runs ($n=6$) yielded 9–33 mCi (0.33–1.2 GBq) starting from 77–179 mCi (2.8–6.6 GBq). To establish suitability for larger scale production, another synthesis was performed ($n=1$) starting with 1.0 Ci (37 GBq) of activity that resulted in 189 mCi (7.0 GBq) of ^{18}F-FDHT after reformulation (32% decay-corrected yield), sufficient for multiple human patient doses. Overall synthesis time was 90 min: synthesis (56 min), purification (20 min), and reformulation of the product for injection (14 min). Specific activity for the validation runs was 1.5 ± 0.1 Ci/μmol (55 ± 4 GBq/μmol) ($n=3$) at the end of formulation using starting activities of 77–92 mCi (2.8–3.4 GBq). The high activity synthesis resulted in a specific activity of 4.6 Ci/μmol (170 GBq/μmol) at the end of formulation. Approximately 5% of the decay-corrected starting activity was lost during reformulation to the sterile filter (4%) and the transfer line (1%), which was determined, after removal of the sterile filter and formulated product vial, by rinsing the pear flask and subsequent fluid lines with acetonitrile. Additional decay-corrected losses during synthesis (e.g., residue in vials, cartridges, transfer lines) were minimal, accounting for $< 10\%$ of the starting activity. Analytical HPLC was first performed on the EtOH/saline solution used for reformulation to determine peaks resulting from solvent effects. Sample chromatograms of this along with the reformulated ^{18}F-FDHT, with and without cold standard, are shown in Fig. 2. All clinical-level QC test results were in full accordance with cGMP specifications in place at MSKCC (Table 2).

4. Discussion

The fluorinated AR-binding ligand ^{18}F-FDHT has great potential for both research and clinical investigations in the management of metastatic prostate cancer. The automated synthesis of ^{18}F-FDHT described herein will allow for routine production of this PET tracer at multiple facilities. With the development of a reagent kit for ^{18}F-FDHT and the use of disposable cassettes, a technician can perform the synthesis quickly and reproducibly. This is a significant advantage over the current, manual production of ^{18}F-FDHT, which requires manual preparation of reagents and a highly experienced production radiochemist to routinely perform the synthesis.

Due to the highly reactive nature of $LiAlH_4$, the reduction step is highly exothermic and, in the manual synthesis, is performed at -78 °C to tame the reactivity of the reducing reagent and also to minimize the formation of undesirable side products. Since cryogenic cooling is not available on commercial radiosynthesizers for ^{18}F-labeled PET tracers, this step has previously hindered automation of the ^{18}F-FDHT synthesis. To avoid the need for cryogenic conditions while utilizing $LiAlH_4$, the reducing agent is slowly added to minimize the rate of heat generation and potential side product formation.

In the reduction of ketone **2** (Scheme 1) with $LiAlH_4$, a potential side product that could occur at increased temperature is the reduction of the C–F bond. Alkyl halides can be reduced by $LiAlH_4$ in ethereal solvents; however, the rates of reduction markedly decrease from iodide to bromide to chloride (I > Br > Cl) and from primary > secondary > tertiary halides, thus defluorination was not expected to be significant (Johnson et al., 1948). In fact, a kinetic study of $LiAlH_4$ reductions at room temperature in THF with various alky halides illustrated that cyclopentyl bromide, which is a more reactive model for the cyclopentyl fluoride **2**, underwent only 11% reduction after 30 min at room temperature and required 24 h to fully reduce (Krishnamurthy and Brown, 1982). The trend in alkyl halide reactivity rates, in addition to the short reaction time of ketone **2** with $LiAlH_4$ (i.e., 20 s), suggests that the C–F bond would be stable under such reaction conditions. As expected, radio-TLC samples taken before and after the $LiAlH_4$ reduction to assess the respective ratios between highly polar radioactive compounds (e.g., free ^{18}F-fluoride) and ^{18}F-fluorinated compounds remained unchanged (Supplementary Fig. S1).

A time course for the $LiAlH_4$ reaction was performed and reduction of ketone **2** was observed (via analytical HPLC) to be complete after 20 s of stirring (Supplementary Fig. S1), and thus the reaction was quenched with an acetone–THF solution 20 s after the $LiAlH_4$ addition. The amount of acetone was optimized to fully quench excess $LiAlH_4$ without causing a large exotherm in the reaction vial. Too much acetone resulted in a rapid exotherm and the formation of significant aluminum salt deposits on the surface of the vial, which, we hypothesized, was the reason for substantial

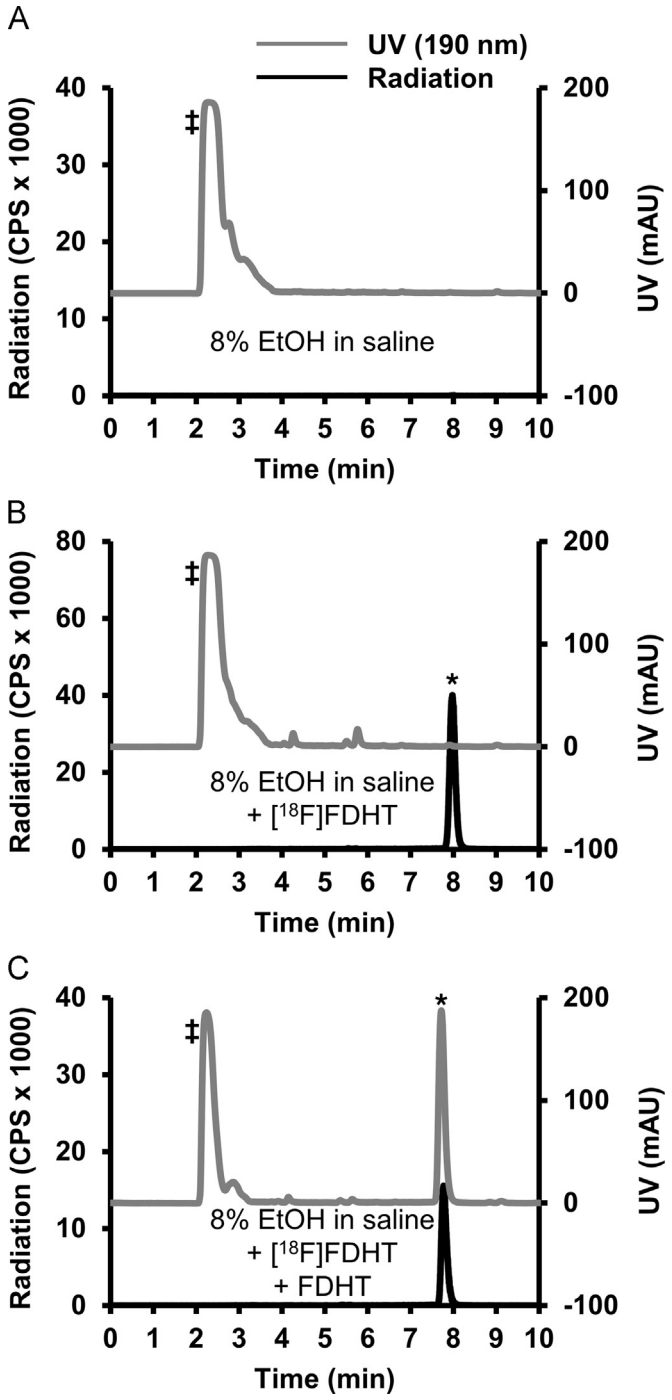


Fig. 2. Analytical HPLC chromatograms from reformulated ^{18}F-FDHT solution. (A) Pure reformulation solvent (‡). (B) Reformulated ^{18}F-FDHT (⁎). (C) Co-injection of ^{18}F-FDHT with ^{19}F-FDHT.

retention of up to 11% of decay-corrected starting activity on the reaction vial. In addition, retention of the desired intermediate **3** on the inside walls of the vial led to inefficient deprotection in the subsequent step due to the inability of the HCl solution to reach the top of the vial; intermediate **3** was observed via analytical HPLC after aqueous extraction (Supplementary Fig. S2) and during the purification of the final product (Supplementary Fig. S3). On the contrary, insufficient acetone left excess $LiAlH_4$ in the reaction mixture resulting in a similar exotherm during the acid-deprotection step. Significantly reduced salt deposition and increased reproducibility of yields were obtained when using 0.10 mL of acetone diluted with 0.25 mL of THF. This ratio was chosen to

Table 2
Clinical level quality control test acceptance criteria routinely used at MSKCC for manual ^{18}F-FDHT synthesis compared to results from this study.

Clinical QC test	MSKCC acceptance criteria	Results of this study
Optical clarity	Clear and particle free	Clear and particle free
pH	5.5–8.0	5.5–6.0
Radiochemical purity	> 95%	98%
Radiochemical identity	Matches retention time of the standard	Matches retention time of the standard
F18 radionuclide identity	Half-life 105–115 min	111 min
Endotoxin level (LAL)	< 5 EU/mL	0.318 EU/mL
Filter integrity	> 50 psig	51 psig
Ethanol content	< 10% (100,000 ppm)	70,000–80,000 ppm
Sterility	No growth in 14 days	No growth in 14 days

maintain a sufficient reagent volume for reliable addition to the reaction vessel and also to maintain the 0.35 mL volume utilized in the original manual synthesis. Gratifyingly, under these conditions, the exotherm was controlled. Visual inspection of the diluted acetone quench revealed minimal splashing of material on the vial, the loss of activity stuck on the reaction vial was notably decreased to < 2%, and the presence of ketal **3** during HPLC purification was substantially lowered (Supplementary Fig. S4).

Additional minor improvements were made to better automate other aspects of the manual synthesis to the ELIXYS radiosynthesizer. For example, the use of 9 mL vs. 15 mL of water was sufficient to transfer product to the HLB cartridge for the aqueous extraction and subsequently rinse the cartridge. Lowering the required water volume decreased the overall synthesis time by minimizing the operations needed to add and transfer the water. Also, the configuration of drying cartridges was modified in order to prevent potential blockage of the fluid pathway from Cassette 1 to Cassette 2. Initial attempts to solely use $MgSO_4$ as done at MSKCC were unsuccessful due to rapid hardening of the drying agent, consequently obstructing the flow. After testing both type and quantity of drying agents, we found that initially using the milder drying agent, Na_2SO_4, removed the majority of water from the organic solution, allowing more rigorous drying via $MgSO_4$ to occur reliably without hindering the flow.

Taken together, these measures enabled complete automation of the synthesis, resulting in a useful RCY. The RCY we obtained was only modestly lower than the RCY of the manual procedure currently performed at MSKCC (16% vs. 22% without decay-correction). Moreover, specific activities greater than 1 Ci/μmol (37 GBq/μmol) were obtained and multiple clinical doses could be produced. This point was validated with a production run performed using 1.0 Ci of starting activity that yielded ^{18}F-FDHT (189 mCi, 7.0 GBq) with increased specific activity (4.6 Ci/μmol, 169 GBq/μmol), sufficient for multiple patient doses. Combined with the formulated product having passed all clinical-level QC requirements, the synthesis is suitable for direct use in clinical production. Much larger starting activities are routinely available from cyclotrons if larger quantities of ^{18}F-FDHT are desired.

With the ELIXYS radiosynthesizer, we have successfully automated the currently utilized ^{18}F-FDHT synthesis with only minor modifications. Recent reports have shown the potential for modification of the conventional synthesis methodology to eliminate the use of $LiAlH_4$ and normal-phase HPLC purification. The primary goal of such changes is to eventually enable a facile translation of the ^{18}F-FDHT synthesis onto other commercial automated synthesizers (e.g., GE TRACERlab™). Alterations to the synthesis protocol have been presented as conference abstracts in which, for example, reverse-phase HPLC purification was implemented to allow for rapid and straightforward reformulation using C18 solid-phase extraction after purification (Nickels et al., 2014). In

addition, the use of $NaBH_4$ as a milder reducing agent was recently optimized in the manual synthesis of ^{18}F-FDHT to avoid cryogenic conditions (Zhou et al., 2014). Under this protocol the reduction step was performed at room temperature in ethanol and was complete after 10 min. However, prolonged stirring of the reaction mixture in the presence of acetone was required to quench excess $NaBH_4$; this critical step avoids significant decomposition of the desired product during the subsequent acid mediated deprotection. While the slow decomposition of $NaBH_4$ in protic solvents to form H_2 gas is an insignificant concern at the time-scale of the reduction, this incompatibility creates a limitation with regard to reagent preparation and automation of this protocol. The requirement to prepare a fresh $NaBH_4$ solution before each synthesis, therefore, limits the practicality of commercially available reagent kits to be provided for the automated synthesis of ^{18}F-FDHT. Furthermore, the low solubility of $NaBH_4$ in alcoholic solvents may result in complications of reagent addition (e.g., clogging) during use in commercial automated synthesizers. An automated synthesis of ^{18}F-FDHT using a $NaBH_4$ reduction method was presented in a recent abstract (Mori et al., 2010), but to our knowledge no peer-reviewed article applying a fully-automated protocol for ^{18}F-FDHT has been previously published. Ultimately, we decided against the use of $NaBH_4$ and reverse-phase HPLC in this study to (1) accelerate translation to an automated protocol by avoiding significant re-optimization of these steps, and (2) demonstrate that $LiAlH_4$ reduction and normal-phase HPLC purification are possible using the ELIXYS radiosynthesizer, further demonstrating the system's versatility. Of course, simplifications or improvements to the ^{18}F-FDHT synthesis protocol could quickly be applied to the ELIXYS radiosynthesizer if desired. Moreover, the automation of a $LiAlH_4$ reaction presented in this study could also be applied to other automated synthesizers, and potentially the synthesis of other PET tracers.

5. Conclusion

The radiosynthesis of ^{18}F-FDHT has been adapted for full automation on the ELIXYS radiosynthesizer resulting in good yields, high purity and good specific activity suitable for clinical use. In addition to the advantages of automation, the use of disposable kits on ELIXYS will enable the creation of reagent kits to simplify the overall protocol and facilitate routine production of ^{18}F-FDHT. Ultimately, we hope that the automated synthesis of ^{18}F-FDHT will be adopted by others and accelerate research by enabling widespread access to this promising PET tracer, potentially aiding in the clinical management of metastatic prostate cancer.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apradiso.2015.05.010.

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